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Epidemiological studies on dermatophilosis in the Caribbean

MARTINEZ (D.), AUMONT (G.), MOUTOUSSAMY (M.), GABRIEL (D.), TATAREAU (A.H.), BARRÉ (N.), VALLÉE (F.), MARI (B.). Études épidémiologiques sur la dermatophilose dans les Antilles. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 323-327

La dermatophilose est une des maladies les plus importantes des ruminants domestiques des îles caraïbes, où la maladie clinique est associée à la présence de la tique *Amblyomma variegatum*. Des études séroépidémiologiques ont été effectuées afin d'éclaircir l'épidémiologie de la maladie dans la région, en faisant particulièrement attention au rôle d'*A.variegatum*. Une banque de 1300 sérums de bovins des Petites Antilles a été examinée par ELISA pour la présence d'anticorps contre *Dermatophilus congolensis*. Il s'est avéré que des animaux séropositifs existent dans des îles non infestées par *A. variegatum*, et où la dermatophilose n'est jamais ou rarement observée. De plus, il n'y avait pas de différence significative entre la prévalence d'animaux séropositifs des zones infestées par la tique et des zones non infestées de la Martinique et de Sainte-Lucie, deux îles partiellement infestées et où la dermatophilose n'est observée que dans les parties infestées par la tique. La prévalence était basse dans les petites îles ayant un climat sec. Ceci confirme les résultats expérimentaux indiquant qu'*A. variegatum* n'est pas indispensable pour la transmission de *D. congolensis*, qui est très répandu dans la plupart des îles. Les concentrations élevées de prostaglandine E2 (entre 151 et 377 ng/ml) et de prostacycline (entre 124 et 134 ng/ml) trouvées dans la salive des femelles d'*A. variegatum*, suggèrent fortement que la tique pourrait favoriser le développement des lésions par une activité immunomodulatrice de sa salive. Néanmoins, malgré un certain succès dans la reproduction de la dermatophilose chez des chèvres simultanément infestées avec des adultes d' *A. variegatum* et scarifiées avec *Dermatophilus*, on n'a pas observé de différence entre des bovins Créole naturellement résistants et des Brahman hautement sensibles, utilisant le même modèle. Les lésions de la dermatophilose sont restées très bénignes sur les animaux des deux races. Après cette expérience, les Brahman ont développé une dermatophilose généralisée après avoir été mis au pâturage, ce qui indique que le rôle respectif des facteurs de risque identifiés comme étant d'importance majeure pour l'expression de la dermatophilose clinique, n'est pas complètement clarifié et demande d'être étudié davantage.

Mots clés : Bovin Créole - Dermatophilose - *Dermatophilus congolensis* - Epidémiologie - Enquête sérologique - Test ELISA - Infection expérimentale - *Amblyomma variegatum* - Salive - Martinique - Sainte-Lucie.

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INTRODUCTION

Dermatophilosis is an exsudative skin disease affecting numerous animal species and man caused by an Actinomycete, *Dermatophilus congolensis*. The microorganism has a worldwide distribution but is of little economic significance in temperate climates. However, severe economic losses occur in domestic ruminants and horses in the tropics (8). *D. congolenis* is not very pathogenic per se and there is no reliable method to reproduce the disease experimentally. Cofactors are necessary for the appearence of the clinical disease among which the breed, the humidity and the bite of arthropods are of primary importance (2). In the Caribbean, a close correlation was noted between the distribution of the tick *Amblyomma variegatum* and the severe extensive forms of the disease (4). In fact, it has long been noted that outbreaks are associated with the presence of this tick (17, 16). In the Antilles, outbreaks of the disease occur soon after the introduction of *A. variegatum* in a new island and in partly infected islands acute dermatophilosis is restricted to those areas where the tick is known to occur (5, 6, 12, 13, 14). In such situations the mortality in susceptible animals may exceed 50 % in the absence of treatment (15, 18) and the disease is a major pathological constraint for farmers. Since 1948, 17 new islands of the Lesser Antilles have become infected by *A. variegatum* (3), and this tick with its associated diseases represents a real threat for the American mainland (1). In the absence of a vaccine, tick control is the most effective method of controlling the disease.

Recently, by using rifampicin resistant strains of *D. congolensis*, it was experimentally shown that *A. variegatum* did not act as a vector of *D. congolensis*. Transmission occurred without the presence of ticks, and high levels of specific antibodies were elicited in goats which did not develop any lesions until adult *A. variegatum* were allowed to feed on them. An extensive dermatophilosis was thus experimentally reproduced leading to the death of experimental goats (10). The specificity of the association between acute dermatophilosis and this particular tick strongly suggests that unknown agents in the tick saliva could favour the development of skin lesions on asymptomatic carrier animals.

In this study we report the results of seroepidemiological surveys which were carried out in order to confirm experimental data on the role of *A. variegatum* in the transmission of *D. congolensis*. In addition, attempts to reproduce

dermatophilosis on cattle and preliminary investigations on the composition of *A. variegatum* saliva were carried out.

MATERIAL AND METHODS

Seroepidemiological surveys

Screening of a bank of sera

A bank of 1397 cattle sera collected during a survey on the distribution of heartwater and *Amblyomma variegatum* in the Caribbean conducted between 1983 and 1984 (4), was screened for the presence of antibodies to *D. congolensis* using an ELISA method (11).

Surveys in Martinique (1989) and in Saint Lucia (1990)

These surveys were conducted to study the relationship between the presence of antibodies to *D. congolensis* in domestic ruminant sera and the presence of clinical lesions of dermatophilosis on animals, along with their infestation by *A. variegatum*. Field studies consisted of a questionnaire-based interview with farmers about farm management, and a visual and physical inspection of animals (sex, age, breed, body score, tick count, extension and severity of dermatophilosis lesions) in selected herds. Two hundred and sixty one cattle sera were collected in 227 farms representing between 2 and 3 % of the overall number of herds in Martinique. Hundred and seventy two farms were visited in Saint Lucia in which 361 heads of cattle (3 % of the herd), 183 sheep (1 %) and 110 goats (1 %) were bled. The farms surveyed were spread all over the islands and the sera were tested by the above-mentioned ELISA method.

Attempts to reproduce extensive dermatophilosis on cattle

Fifteen Brahman and 15 Creole cattle were given 3 successive inoculations of *D. congolensis* with or without infestation with *A. variegatum* at monthly intervals. Animals were divided into 3 groups. In group 1 composed of 3 Brahman and 3 Creole, animals were infested with 30 pairs of adult *A. variegatum*. In group 2, 8 Brahman and 8 Creole zebus were simultaneously infested with 30 pairs of adult ticks and scarified with the R2 rifampicin resistant strain of *D. congolensis* (9). Group 3 consisted of 4 Brahman and 4 Creole animals scarified with *D. congolensis* in the absence of ticks. A Guadeloupe strain of *A. variegatum* reared in our laboratory on experimental goats and maintained at 25 °C and 90 % relative humidity for several generations was used for infestation of animals. Ticks were confined to hosts in bags glued to the skin of the flank with adhesive. Female ticks were applied 7 days after the fixation of males. Inoculation with *D. congolensis* was carried out by swabbing 5 ml of a broth culture containing approximately 10^7 cfu/ml on each of four 100 cm^2 skin areas of the rump previously shaved and defatted with acetone. Skin lesions were scored twice a week (10) and blood collected at weekly intervals on each animal. At the end of the experiments, animals were put into the fields in the same farm. The infestation by *A. variegatum* (tick count), the appearence and evolution of lesions of dermatophilosis were recorded each week for 3 months.

Dosage of prostaglandins in tick saliva

To confirm the hypothesis raised in previous studies (10) of an immunomodulation induced in the host by the tick bite, preliminary investigations on the presence of immunosuppressive components in the tick saliva were carried out. In particular, the presence and the amount of prostaglandin E2 and I2 which are known to have potent immunosuppressive activities, were determined in tick saliva using a competitive enzyme immunoassay (Cayman chemical). The test was performed according to the instructions of the manufacturer.

RESULTS AND DISCUSSION

The prevalence of cattle with antibodies to *D. congolensis* in relation with the presence of *A. variegatum* in the Lesser Antilles (sera from 1983-84) is summarized in table I. The seroprevalence was high in all islands infested by *A. variegatum*, but also in Cariacou, Saint Vincent and Tortola where this tick was not reported to occur. This is in agreement with the experimental results of MARTINEZ et al. (10) demonstrating the high levels of transmission of *D. congolensis* in the absence of ticks. The prevalence of seropositive cattle was low in the small dry *Amblyomma* free islands of Anegada, Jost van Dyck, Virgin Gorda, Saba and Saint Eustachius suggesting that the efficacy of transmission as quantified by the seroprevalence, was decreased in dry conditions. Martinique and Saint Lucia were selected to conduct a field study because they were only partly infested by *A. variegatum* and therefore provided a unique situation to study the association between this tick and *D. congolensis*. The percentages of seropositive animals were not significantly different between areas infested or not by *A. variegatum* (table II), confirming that this tick has no significant role in transmitting the microorganism. Moreover, the prevalence of animals with antibodies was very high, indicating that

TABLE I Seroprevalence of dermatophilosis in cattle in the Lesser Antilles (sera from Burridge et al., *1984).*

Island	Number of sera	Positive sera n (%)	*Amblyomma variegatum*
Virgin Islands :			
Anegada	19	3 (16)	–
Jost van Dyck	11	2 (18)	–
Tortola	141	48 (34)	–
Virgin Gorda	23	1 (4)	–
Anguilla	67	15 (22)	–
Saint Martin	72	54 (75)	+
Saba	27	3 (11)	–
Saint Eustachius	31	3 (10)	–
Saint Kitts	61	53 (87)	+
Antigua	199	97 (49)	+
Montserrat	155	91 (59)	+
Dominique	108	90 (83)	+*
Saint Lucia	82	54 (66)	+*
Saint Vincent	181	108 (60)	–
Cariacou	58	47 (81)	–
Barbados	108	41 (38)	+*
Les Saintes	54	1 (2)	–

* A. variegatum *is established but with limited distribution.*

TABLE II Seroprevalence of dermatophilosis on domestic ruminants in Martinique and Saint Lucia.

Saint Lucia		n	% positive sera
Cattle	non infested area	240	60[a]
	infested area	121	56[a]
	total	361	59
Sheep	non infested area	121	53[a]
	infested area	62	63[a]
	total	183	56
Goats	non infested area	75	73[b]
	infested area	35	86[b]
	total	110	77
Martinique		n	% positive sera
Cattle	non infested area	99	62[a]
	infested area	162	71[a]
	total	261	67

In the same column and the same island, numbers with different superscript letters are significantly different ($P < 0.05$).

the transmission was very efficient. However, clinical cases of dermatophilosis were reported in tick infested areas only. The possibility that the tick could favour the development of scabs through an immunomodulating activity of its saliva instead of a vector capacity, was corroborated by the high levels of PGE2 (151 to 377 ng/ml) and PGI2 (124 to 134 ng/ml) found in its saliva. These agents have potent immunomodulating activities (7) and might have influenced the course of the disease. However, their activity is restricted to those areas where they are secreted. It is thus unlikely that they have a great importance in the induction of lesions distant from the tick bite. In the West Indies, it was found that 90 % of infected cattle had lesions on the back where less than 1 % of the ticks attach (2, 14) suggesting that another mechanism than prostaglandins is involved in the development of such lesions.

The identification of the major risk factors (presence of *A. variegatum,* humidity, susceptible breed) in the development of extensive dermatophilosis and the success in reproducing the disease on Creole goats using an experimental model taking into account all these factors (10), led us to use the same model to reproduce the disease on cattle. The influence of the breed was investigated by comparing a highly susceptible (Brahman zebu) and a highly resistant Creole cattle from Guadeloupe) breed of cattle. There was no development of scabs in groups of animals inoculated with *A. variegatum* alone (group 1). In contrast, on all animals inoculated with *D. congolensis* (groups 2 and 3), scabs developed at the sites of inoculation from where the rifampicin resistant strain could be isolated. The score lesion was increased by the presence of ticks in Creole but not in Brahman cattle (figure 1). Surprisingly, Creole cattle had higher score lesions than Brahman cattle. However, the reproduction of an extensive dermatophilosis failed in both breeds : the lesions remained very mild and located at the sites of inoculation. Differences in score lesion could not be attributed to a higher infestation of Creole cattle by ticks since there was no significant difference between breeds in the percentage of female ticks which attached and engorged, except at the first infestation (figure 2). All Brahman animals having experienced a severe dermatophilosis episode several months before the experiment (15), it was decided to verify the very unlikely hypothesis that they had become protected against the disease. The 30 heads of cattle were put on an infested pasture of the farm where the experiment had been conducted. All animals become infested by *A. variegatum* a few days after their introduction on the pasture. The tick burden varied from 2 to 42 adult *A. variegatum* per animal. As expected, none of the Creole cattle developed any lesion, whereas all Brahman zebus but one started developing scabs within 2 weeks after their introduction on the pasture. The lesions became generalized within 2 to 6 weeks in 9 out of 15 animals which had to be treated with long acting Terramycin®. Five animals with only localized lesions were not treated, and one animal did not develop any lesion during the 3 months period of monitoring in the field. As in the experiments conducted on goats (10), the strain isolated from the scabs was a non rifampicin resistant field strain of *Dermatophilus.* Whether this strain was already present on the animals or was transmitted in the field was not elucidated.

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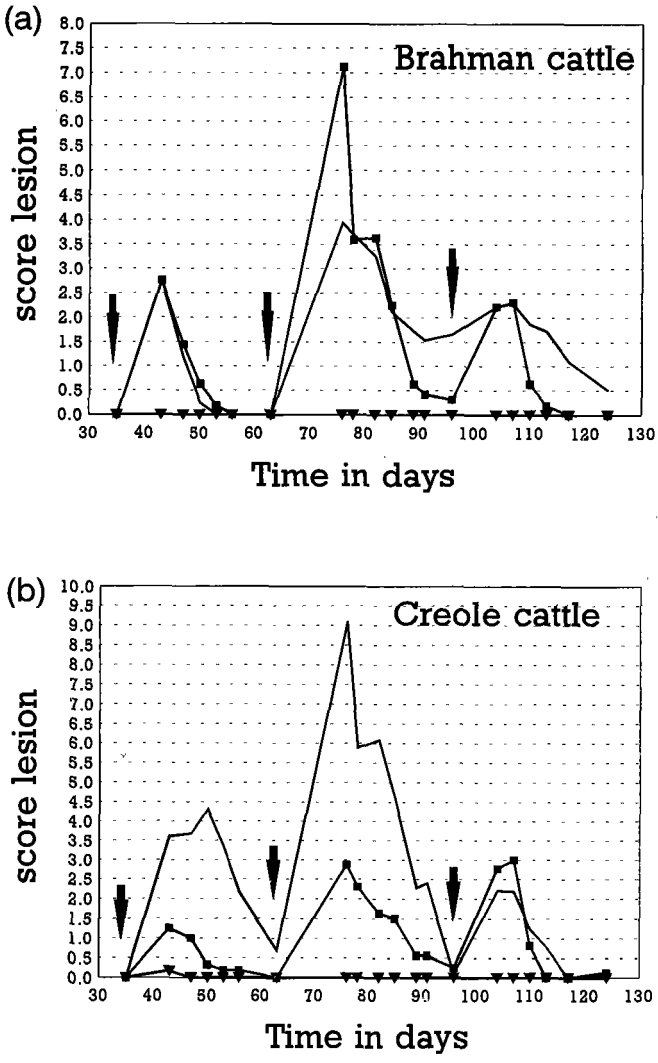

Figure 1 : Score lesion of 15 Brahman (figure a) and 15 Creole (figure b) cattle experimentally infested with 30 pairs of adult Amblyomma variegatum *without* Dermatophilus congolensis *(Δ) scarified with the R2 strain of* D. congolensis *in the absence of ticks (□), or simultaneously scarified with* D. congolensis *and infested with 30 pairs of* A. variegatum *(–). Each infestation is represented by an arrow.*

The failure to reproduce the disease could have been due to a low pathogenicity of the R2 strain, to differences between wild ticks and ticks reared in the laboratory, or to the absence of a badly identified environmental factor. Except for *Amblyomma* and *Dermatophilus* strains, the only difference in the management of animals were that cattle were maintained in shaded paddocks during the experiment and exposed to direct sunlight in the pasture. Although sunrays alone did not induce the development of scabs (the same Brahman animals maintained under the sun in tick free paddocks did not develop any lesions), they might have acted synergistically with ticks.

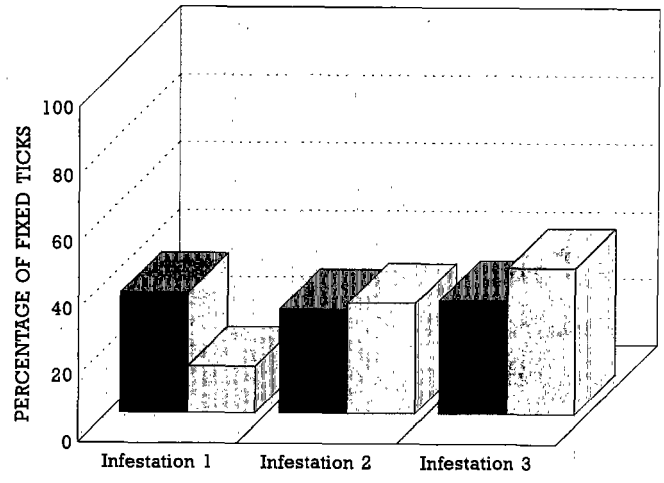

Figure 2 : Percentage of adult Amblyomma variegatum *which attached and engorged on Brahman (▒) and Creole (■) cattle during 3 successive infestations at 1 month interval. Each animal was infested with 30 pairs of adult ticks per infestation.*

In conclusion, these epidemiological data confirm that *A. variegatum* does not play a significant role in the transmission of *D. congolensis* and its major influence in the induction of scabs. However, it appeared that despite some previous success in reproducing dermatophilosis on goats experimentally, the respective role of the risk factors identified as being of major importance for the expression of clinical dermatophilosis is not clarified and needs further investigations.

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MARTINEZ (D.), AUMONT (G.), MOUTOUSSAMY (M.), GABRIEL (D.), TATAREAU (A.H.), BARRÉ (N.), VALLÉE (F.), MARI (B.). Epidemiological studies on dermatophilosis in the Caribbean. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 323-327

Dermatophilosis is one of the most important diseases of domestic ruminants in the Caribbean islands where the clinical disease has been shown to be associated with the presence of the tick *Amblyomma variegatum*. Seroepidemiological studies were conducted to clarify the epidemiology of the disease in the region with a particular attention paid to the role of A. variegatum. A bank of 1300 cattle sera from the Lesser Antilles was screened by ELISA for the presence of antibodies to *Dermatophilus congolensis*. It appeared that seropositive animals do exist in islands non infested by *A. variegatum* and where dermatophilosis is never or seldom seen. Moreover, there was no significant difference in prevalence of seropositive animals between tick-infested and non infested areas of Martinique and Saint Lucia, 2 islands partly infested by the tick, and where dermatophilosis is only seen in tick-infested areas. Prevalence was low in small islands with a dry climate. This confirms experimental data showing that *A. variegatum* is not necessary for the transmission of *D. congolensis* which is widespread in most of the islands. High concentrations of prostaglandin E2 (between 151 and 377 ng/ml) and prostacyclin (between 124 and 134 ng/ml) found in the saliva of females *A. variegatum* strongly suggest that the tick could favour the development of the lesions through an immunomodulating activity of its saliva. However, despite some success in reproducing dermatophilosis on goats simultaneously infested with adult *A. variegatum* and scarified with *Dermatophilus*, no difference was observed between naturally resistant Creole cattle and very susceptible Brahman animals using the same model. The lesions of dermatophilosis remained very mild on animals of both breeds. After the experiment, the Brahman animals put onto the field developed an extensive dermatophilosis indicating that the respective role of the risk factors identified as being of major importance for the expression of clinical dermatophilosis is not fully clarified and needs further investigations.

Key words : Creole cattle - Dermatophilosis - *Dermatophilus congolensis* - Epidemiology - Serological survey -ELISA test - Experimental infection - *Amblyomma variegatum* - Saliva - Martinique - Saint Lucia.

MARTINEZ (D.), AUMONT (G.), MOUTOUSSAMY (M.), GABRIEL (D.), TATAREAU (A.H.), BARRÉ (N.), VALLÉE (F.), MARI (B.). Estudios epidemiológicos sobre la dermatofilosis en el Caribe. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 323-327

La dermatofilosis es una de las enfermedades más importantes en las Islas del Caribe, donde la enfermedad clínica se ha asociado a la presencia de garrapatas de *Amblyomma variegatum*. Se han llevado a cabo estudios sero-epidemiológicos para aclarar la epidemiología de la enfermedad en la región y en particular el papel de *A. variegatum*. Un banco de 1300 sueros bovinos provenientes de las Antillas Menores fue sometido a un monitoreo por ELISA, con el fin de detectar la presencia de anticuerpos contra *Dermatophilus congolensis*. Según los resultados, existen animales seropositivos en islas libres de A.variegatum y en las cuales la dermatofilosis se ha presentado raramente o nunca. Aún más, no se encontró diferencia significativa en la prevalencia de animales seropositivos entre las áreas infestadas con la garrapata y las no infestadas de Martinica y Santa Lucia, islas parcialmente infestadas y en donde la dermatofilosis aparece solamente en las zonas infestadas. La prevalencia fue menor en las pequeñas islas de clima seco. Esto confirma los datos experimentales, según los cuáles *A. variegatum* no es esencial para la transmisión de *D. congolensis*, que se encuentra diseminado en la mayoría de las islas. Se encontraron concentraciones elevadas de prostaglandina E2 (entre 151 y 377 ng/ml) y prostaciclina (entre 124 y 134 ng/ml) en la saliva de hembras de *A. variegatum*. Esto sugiere que la garrapata podría favorecer el desarrollo de lesiones mediante una actividad de modulación inmunológica de la saliva. Sin embargo, a pesar de un ligero éxito en la reproducción de la dermatofilosis en cabras inoculadas simultaneamente con adultos de *A. variegatum* y con *Dermatophilus*, no se encontró diferencia entre la resistencia natural del ganado "Créole" y la de los animales Brahman, altamente susceptibles. Las lesiones de dermatofilosis fueron leves en los animales de ambas razas. Después del experimento, los animales Brahman que fueron sometidos a condiciones de campo, desarrollaron una dermatofilosis extensa. Esto sugiere que el papel atribuído a los factores de riesgo catalogados como importantes para la aparición de la dermatofilosis clínica, no es aún claro y necesita investigaciones ulteriores.

Palabras claves : Bovino Creole - Dermatofilosis - *Dermatophilus congolensis* - Epidemiologia - Encuesta serologica - Test ELISA - Infección experimental - *Amblyomma variegatum* - Saliva - Martinica - Santa Lucia.